

## Circadian Rhythms in Locomotor Activity of the Hagfish, *Eptatretus burgeri* III. Hypothalamus: a Locus of the Circadian Pacemaker?

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**ABSTRACT**—By recording the locomotor activity rhythms of hagfish in which partial ablations in brain were made, this work was designed to determine the location of the circadian pacemaker. The characteristic rhythms were maintained in the absence of the optic tectum, but were lost in animal lacking the telencephalon or diencephalon. The telencephalon-diencephalon unit was more finely dissected for more precise localization. The rhythm still occurred without the upper or the lateral parts of this anatomical unit. Transecting the middle of the telencephalon had no effect on the rhythms displayed. These findings suggest that a crucial part (circadian pacemaker?) for the fish to be rhythmic may be in the ventromedial part of telencephalon-diencephalon, the hypothalamus, the frontal part of which extends under the telencephalon.

### INTRODUCTION

The authors have demonstrated a clear circadian rhythm of the locomotor activity of the hagfish, *Eptatretus burgeri*, using an infra-red light-photocell system [1]. Attempts to determine the localization of the circadian pacemaker by surgical ablation have shown that the pacemaker may be in the brain anterior to the medulla oblongata [2]. In the present study, more precise examination was undertaken to identify more precisely the part of brain where the circadian pacemaker may be situated.

### MATERIALS AND METHODS

All surgery was performed while the hagfish were lightly anesthetized by MS-222. Fixing the animal on a plastic stage, the skin and the fibrous connective tissue covering the brain were cut longitudinally along the median axis. Each part of the brain was removed with a pair of scissors. The connective tissue was replaced as it was originally

and the skin was sewn together. The operated animals were kept in a large aquarium under 12L:12D for one week prior to recording the activity in the experimental aquaria. The method and procedure for recording the activity of the animal have been described in the previous papers [1, 2]. The existence of locomotive rhythm was detected by observation of the distribution of activity records.

### RESULTS

Figure 1 shows diagrammatically the locality of the brain where the surgical cuts were made. The effect of each type of operation on the locomotor activity is summarized in Table 1.

Animals without the optic tectum displayed a nocturnal rhythm under 12L:12D, and a free-running rhythm in constant darkness (Fig. 2).

Without either the telencephalon and anterior part of hypothalamus, or the diencephalon, activity was not rhythmic, but was irregularly intermittent under 12L:12D or under constant darkness (Figs. 3 and 4).

When the dorsal part of the telencephalon-diencephalon was cut out horizontally to the

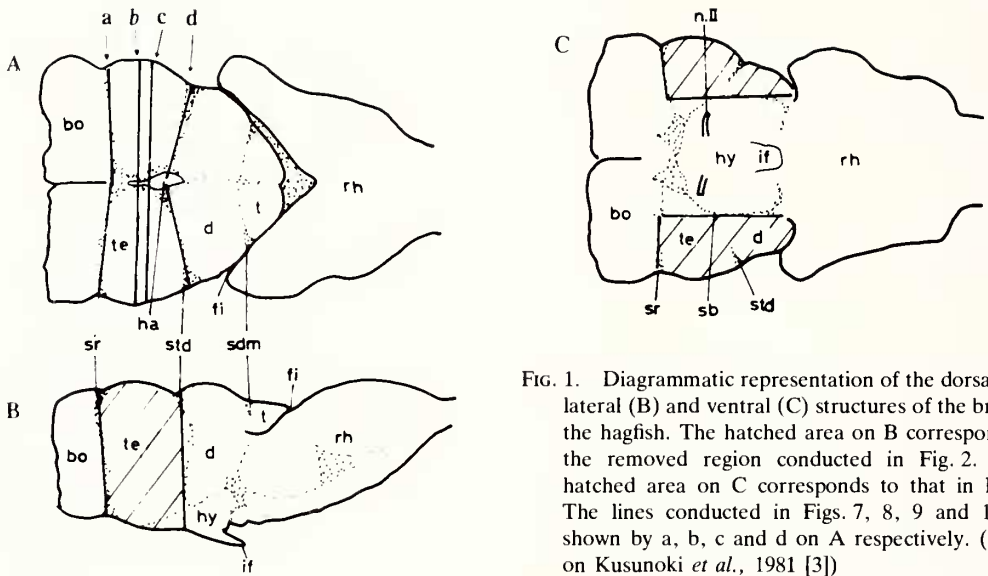


FIG. 1. Diagrammatic representation of the dorsal (A), lateral (B) and ventral (C) structures of the brain in the hagfish. The hatched area on B corresponds to the removed region conducted in Fig. 2. The hatched area on C corresponds to that in Fig. 6. The lines conducted in Figs. 7, 8, 9 and 10 are shown by a, b, c and d on A respectively. (Based on Kusunoki *et al.*, 1981 [3])

TABLE 1. Surgical operations in brain and their effects on locomotor activity rhythm in the hagfish

Removed parts and line-cuts in brain	Specimens tested, number	Free-running rhythm in constant darkness	Nocturnal rhythm in 12L:12D
telencephalon + frontal part of hypothalamus	8	—	—
diencephalon	9	—	—
optic tectum	3	+	+
dorsal part of (telencephalon + diencephalon)			
1/4	2	+	+
1/2	3	+	+
3/5	2	+	+
lateral halves of (telencephalon + diencephalon)	5	+	+*
line-cut at			
a	1	+	+
b	2	+	+
c	2	—	—
d	1	—	—

See Fig. 1 for surgical operations.

+; rhythm positive.

—; rhythm negative.

\*+ the activity is not confined to the dark period.

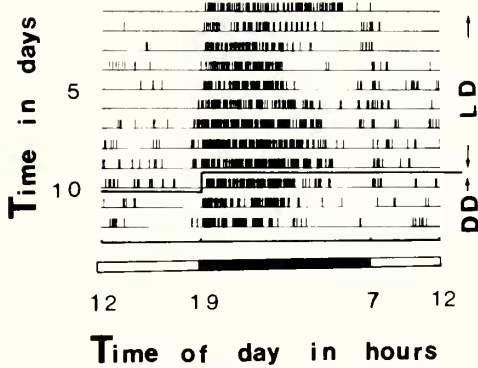


FIG. 2. Locomotor activity of the hagfish without the optic tectum kept under 12L:12D (7:00–19:00 light, 19:00–7:00 dark) and in constant darkness. The activity is indicated by the vertical marks on the time line. The operated hagfish shows activity in the dark period under 12L:12D, and exhibits, in constant darkness, the free running rhythm whose length is about 24 hr.

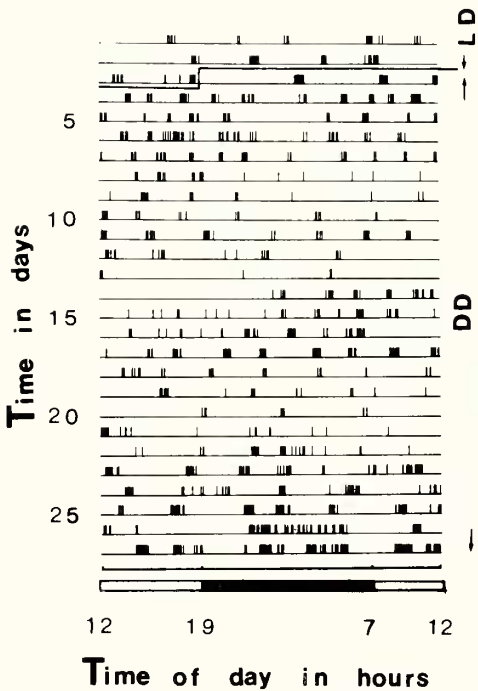


FIG. 3. Locomotor activity of the hagfish without the telencephalon (including the frontal part of hypothalamus) (see Fig. 1B). The operated hagfish shows intermittent activity both in 12L:12D and in constant darkness. This is suggestive of losing the rhythm without the tissues. The peripheral lighting conditions are the same as that explained in Fig. 2, and the following figures (Figs. 4–10) are also in the same.

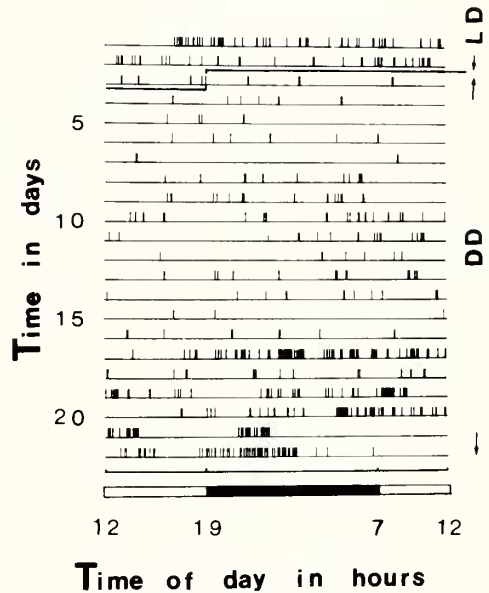


FIG. 4. Rhythm-negative activity without the diencephalon.

depths of 1/4, 1/2 or 3/5, in all cases, the nocturnal rhythms appeared under 12L:12D and free running rhythms appeared under constant darkness (Fig. 5).

In absence of the lateral halves of the telencephalon-diencephalon, the rhythm appeared both under 12L:12D and under constant darkness. However, in the 12L:12D, the activity extended beyond the dark period and occurred even in the light period (Fig. 6). This phenomenon is similar to that in the eye ablation experiment (Kabasawa and Ooka-Souda, unpublished).

After lineal cuts across the full depth of the brain both at the border between the bulbus olfactorius and the telencephalon, and at the middle of the telencephalon, there was motor activity in the dark period under 12L:12D, and the activity pattern showed a free-running rhythm under constant darkness (Figs. 7 and 8). The cuts both anterior to the posterior large habenula and at the border between the telencephalon and the diencephalon caused intermittent activity both under 12L:12D and under constant darkness (Figs. 9 and 10).

From the results described above, it appears that the hypothalamus plays an important part in

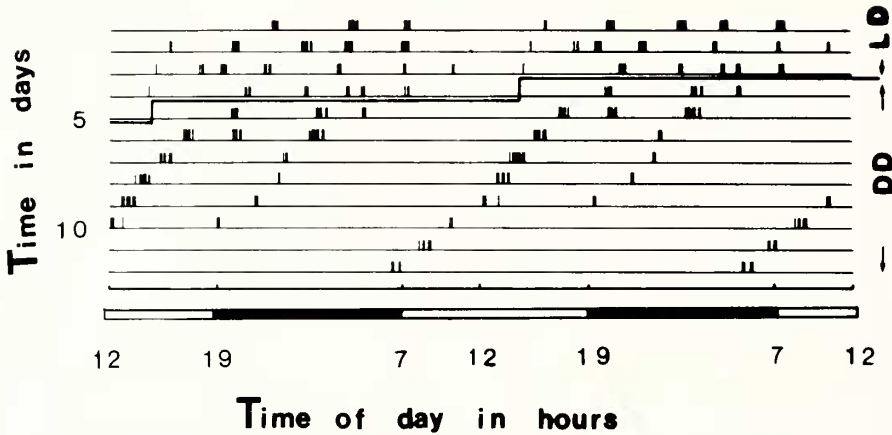


Fig. 5. Rhythm-positive activity without the upper part (3/5) of telencephalon-diencephalon. The original record is plotted twice.

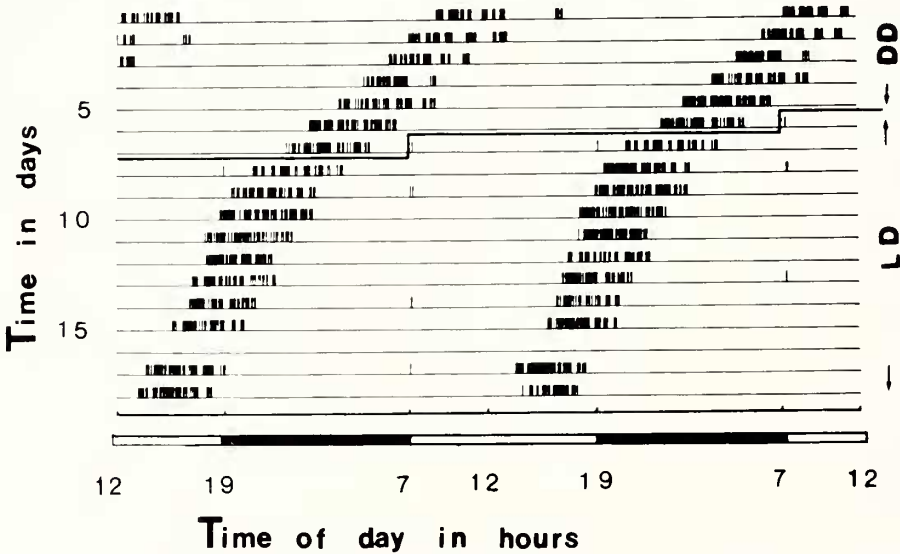


Fig. 6. Rhythm-positive activity without the lateral halves of the telencephalon-diencephalon (see Fig. 1C). In this case, the activity shows a free-running rhythm not only in constant darkness but also in 12L:12D.

circadian locomotor activity system, and it is possibly the locus for the circadian pacemaker function. As shown in Figure 1, the hypothalamus is located in the ventromedial part of the telencephalon-diencephalon and anteriorly it extended as far as the midline of the telencephalon. The results of the transectional experiments can be interpreted as meaning that the hypothalamus may include a circadian pacemaker.

## DISCUSSION

There are many published reports concerning the localization of the circadian pacemaker in vertebrates. The suprachiasmatic nuclei of the rat [4], the pineal body of the chick [5], both the suprachiasmatic nuclei and the pineal body of the house sparrow [6, 7] and the pineal body of the lamprey [8] all have been proposed to be the sites

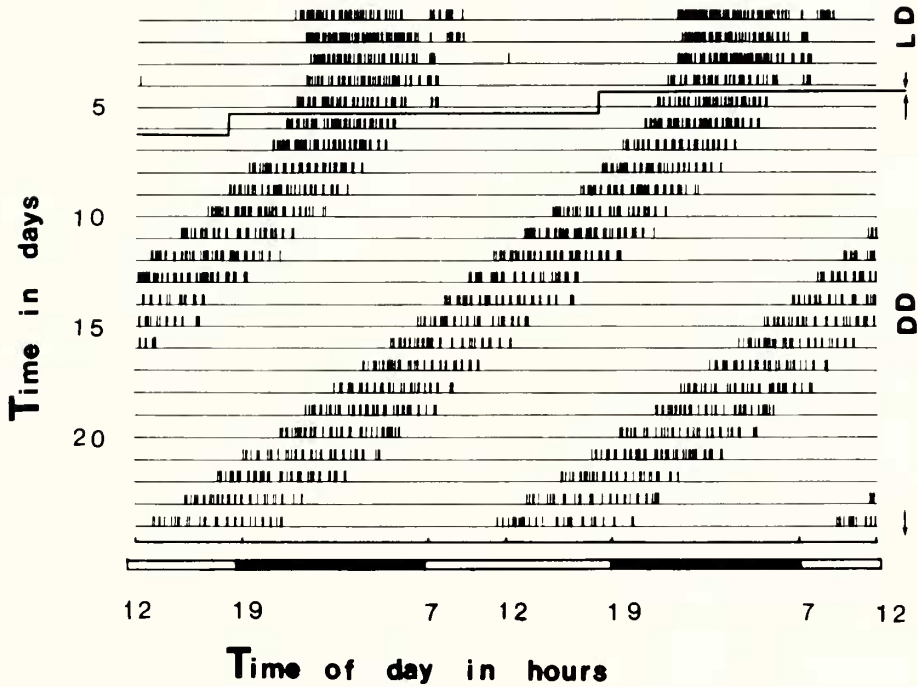


Fig. 7. Rhythm-positive activity with cutting across the border between the bulbus olfactorius and the telencephalon (see Fig. 1A, line a).

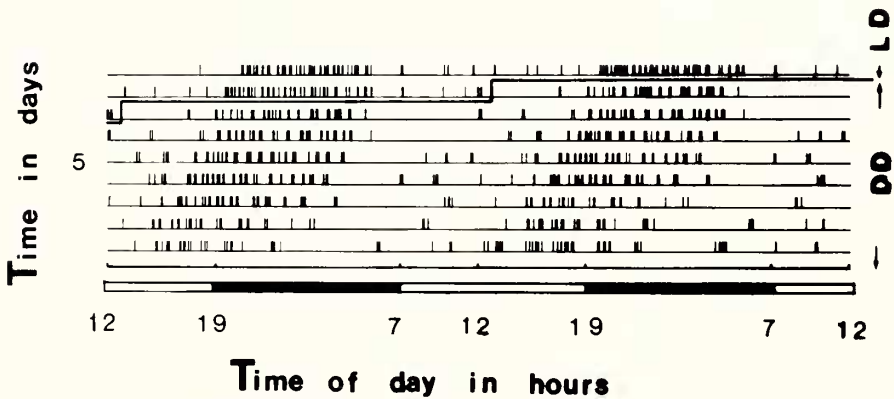


Fig. 8. Rhythm-positive activity with cutting across the middle of telencephalon (see Fig. 1A, line b).

of pacemaker function.

It has been reported that the hagfish has no pineal body [9]. In hagfish in which the dorsal part (3/5) of diencephalon was removed there still was a circadian rhythm in locomotor activity. Thus even if there was a pineal-equivalent tissue in the dorsal thalamus it would not seem to have a pacemaker

function. It is rare in lower vertebrates that the pacemaker is proposed to be situated in the hypothalamus. Continued efforts toward more precise localization of the hagfish circadian pacemaker in the hypothalamus using electrode lesion methods are in progress by the authors.

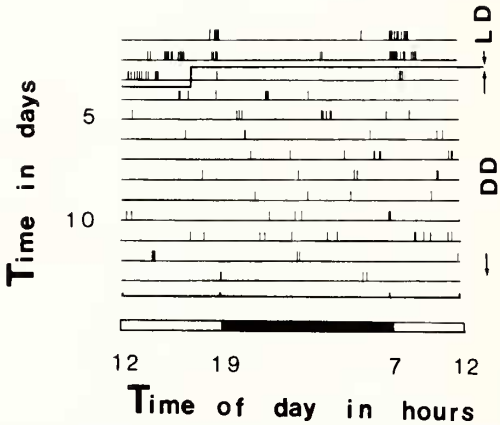


FIG. 9. Rhythm-negative activity with cutting across the front of the posterior habenula (see Fig. 1A, line c).

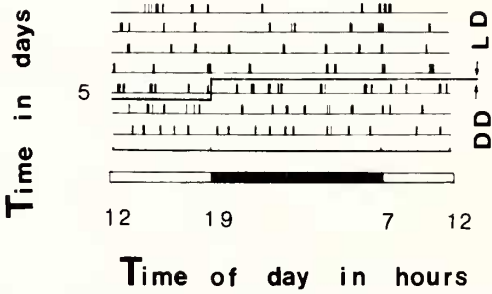


FIG. 10. Rhythm-negative activity with cutting across the border between the telencephalon and the diencephalon (see Fig. 1A, line d).

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